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In vitro distribution of ketoprofen enantiomers in articular tissues of osteoarthritic patients

F. Lagrange [a], T. Fabre [b], F. Péhourcq [a], A. Durandeau [b], B. Bannwarth [a,*]

[a] *Laboratoire de Therapeutique, Department of Clinical Pharmacology and Therapeutics EA525, Université Victor Segalen & Groupe Hospitalier Pellegrin, BP 38, 146 rue Leo Saignat, 33076 Bordeaux Cedex, France*
[b] *Department of Orthopaedics and Traumatology, Université Victor Segalen & Groupe Hospitalier Pellegrin, 33076 Bordeaux Cedex, France*

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Abstract

The distribution of ketoprofen enantiomers in joint tissues was studied as a function of their relative tissular affinities using the multi-chamber distribution dialysis system described by Bickel et al. Selected off-cuts of synovial membrane, joint capsule, cartilage and ligament were obtained from ten patients suffering from osteoarthritis of the knee ($n = 3$) or hip ($n = 7$). Sörensen solution (4 ml) spiked with racemic ketoprofen (2 μg ml^{-1}) was dialysed against 1 ml of the four solutions of tissue homogenates (0.4 g ml^{-1}). Ketoprofen enantiomers were quantified in buffer and tissue solutions by high-performance liquid chromatography. The distribution of ketoprofen enantiomers in the Bickel's multi-compartment model indicated that there was a non-stereoselective affinity of ketoprofen enantiomers for their potential target tissues. Despite the interindividual variability in articular tissues, the concentrations ($\pm$ S.D.) of *R*- and *S*-ketoprofen were significantly higher in synovial membrane (8.69 (4.76) μg g^{-1} for *S*, 9.14 (5.57) μg g^{-1} for *R*), joint capsule (5.71 (2.49) μg g^{-1} for *S*, 5.49 (2.62) μg g^{-1} for *R*) and ligament (6.28 (3.61) μg g^{-1} for *S*, 6.40 (3.64) μg g^{-1} for *R*) than in articular cartilage (3.67 (1.75) μg g^{-1} for *S*, 3.70 (1.67) μg g^{-1} for *R*). There were no significant differences in the distribution of *R*- and *S*-ketoprofen between the solutions of joint capsule, synovium and ligament tissues. These data may be related to differences in ketoprofen affinity for the different constituents of joints. This in vitro distribution profile is similar to that reported in vivo for other non-steroidal anti-inflammatory drugs.

Keywords: Ketoprofen enantiomers; Articular distribution; Tissue binding; Joint; NSAID

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) produce analgesia by acting both centrally and peripherally within the damaged tissues [1]. Their anti-inflammatory activity seems to be related to the concentrations achieved in inflamed

* Corresponding author. Tel.: +33-5-575-71666; fax: +33-5-569-30407.
E-mail address: bernard.bannwarth@thera.u-bordeaux2.fr (B. Bannwarth).

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tissues [2], possibly as a result of inhibition of the production of inflammatory prostaglandins via cyclo-oxygenase-2 (COX-2) inhibition [3]. Interestingly, the expression of COX-2 was shown to be upregulated in the synovium of inflammatory rheumatic diseases [4]. A slight increase in COX-2 expression was also found in the synovial membrane of degenerative osteoarthritis [4]. Both ligaments and joint capsules appear also to be significant sources of pain in osteoarthritis [5]. Furthermore, COX-2 expression has been identified in human osteoarthritis affected cartilage [6]. Ketoprofen is a chiral 2-arylpropionic acid NSAID that is mainly marketed as a racemate, an equimolar mixture of two enantiomers, $R(-)$ and $S(+)$. The latter is a potent non-selective COX-1 and COX-2 inhibitor, while the former is virtually devoid of such property [7]. Considering these data, the distribution of ketoprofen enantiomers was studied in joint tissues using the multi-chamber distribution dialysis system described by Bickel et al. [8].

2. Materials and methods

2.1. Chemicals and reagents

Racemic ketoprofen (KP), internal standard (indomethacin), L-leucinamide and ethyl chloroformate were purchased from Sigma Chemical (St Louis, MI, USA). All chemicals were of analytical reagent or high-performance liquid chromatographic (HPLC) grade. Deionized water, purified in a Milli-Q™ system (Millipore, Bedford, MA, USA) was used throughout the study.

2.2. Apparatus and chromatographic conditions

Mechanical grinding of tissues in liquid nitrogen was carried out in a SPEX mechanical freezer-grinder (model 6700230; Spex Industries Inc., Edison, NJ, USA). The pH was measured with a HI1131 electrode and a HI9318 pH-meter (Hanna Instruments Ltd, Leighton Buzzard, UK). The tissue homogenate present in each tissue solution was vacuum dried using a Vacuum Pump (Pascal, Alcatel®, France).

The HPLC was performed with a 717 plus automatic injector, a M510 pump (Waters™ Assoc., Milford, MA, USA), a UV-1000 Model Ultraviolet detector and a Datajet integrator (Thermo™, San Jose, CA, USA). A UV6000LP SpectraSYSTEM® photodiode-array detector in combination with PC1000 and Spectacle software (Thermo™, San Jose CA, USA) achieved spectral resolution of peaks.

2.3. Ketoprofen assay

The free and total concentrations of (*R*)- and (*S*)-ketoprofen were measured in buffer and tissue homogenates in their corresponding compartments after dialysis using a published HPLC method [13,14]. Briefly, the acidified samples were extracted in dichloromethane. After evaporation of the organic layer, the (*S*)- and (*R*)-ketoprofen were derivatized with a chiral amine (0.1 M L-leucinamide in triethylamine–methanol, 0.014:10 v/v) after addition of ethyl chloroformate (60 mM in acetonitrile) as a coupling reagent. The former diastereoisomeric amides were chromatographed (injection volume, 35 μl) at ambient temperature on a reversed-phase column (Kromasil™ C18, 5 μm, 250 × 4.6 mm i.d.; Hypersil, Cheshire, UK). The mobile phase consisted of phosphate buffer (0.06 M KH_2PO_4–acetonitrile–triethylamine, 51:49:0.1 v/v/v) pumped at a flow rate of 1.8 ml min^{-1}. The retention times for (*R*)-ketoprofen, (*S*)-ketoprofen and indomethacin were 5.7, 6.6 and 12.8 min, respectively. Non-stereoselectivity in the extraction and or derivatization steps has already been demonstrated [15]. The enantiomer extraction was achieved in the different tissue solutions with an efficiency greater than 86%. Since it can be assumed that NSAIDs metabolism does not take place within the joint [16], ketoprofen metabolites were not studied.

2.4. Tissue sampling

Synovium, joint capsule, ligament and cartilage samples (0.7–3 g each) were taken from ten patients (four males, six females), aged 35–85 years, who underwent total arthroplasty of the hip ($n = 7$) or knee ($n = 3$) for disabling osteoarthritis. All

patients gave their informed consent to participate in this in vitro study. None had received an NSAID treatment for at least 2 weeks before surgery. Tissue samples were immediately washed free of surface blood with physiological saline, blotted on filter paper, and weighed. They were stored at −80 °C. Mechanical grinding of tissues in liquid nitrogen was carried out as described previously [9]. For each patient, one aliquot of the resulting fine tissue powder was homogenized (0.4 g ml^{-1}) in Sörensen's phosphate buffer (pH 7.4, 0.067 M). One aliquot of each solution (100–250 μl) was vacuum-dried to determine the weight of dried tissue present in each tissue solution.

2.5. The multi-chamber distribution dialysis system

Bickel et al. [8] developed a competitive distribution dialysis model allowing a drug to be distributed between different binders simultaneously (Fig. 1). The system consisted of two Teflon chambers (Fig. 1A,B) separated by a natural cellulose membrane with a 5000 Da cut-off (Diachema A.G., Ruskliken, Switzerland). An undivided compartment containing 4 ml conventional Sörensen phosphate buffer (pH 7.4, 37 °C, 0.067 M) spiked with 2 μg ml^{-1} racemic ketoprofen faced the four compartments of the opposite half-cell. The concentration of racemic ketoprofen (2 μg ml^{-1}) used corresponded to the maximum concentration observed in synovial fluid (1.95 μg ml^{-1}) in patients who where given a single intramuscular dose of 100 mg racemic ketoprofen [10]. Synovial fluid contained virtually identical concentrations of the individual enantiomers in these patients [11].

The four compartments contained 1 ml homogeneous solutions of joint capsule, synovium, cartilage or ligament tissues (0.4 g ml^{-1}). The multi-compartment cell was dialysed at 37 °C with a rotative Dianorm® equilibrium dialyser (Dianorm Geräte, München, Germany) at a speed of 20 r.p.m. for 4 h. During that time, no significant water shift occurred. The distribution of ketoprofen enantiomers was studied at 3 and 4 h using homogenates drawn from a unique patient. A prerequisite indicated a stable distribution with no subsequent redistribution between the tissue homogenates [8]. The absence of drug adsorption on membrane or cell surfaces had been verified in an earlier experiment [12].

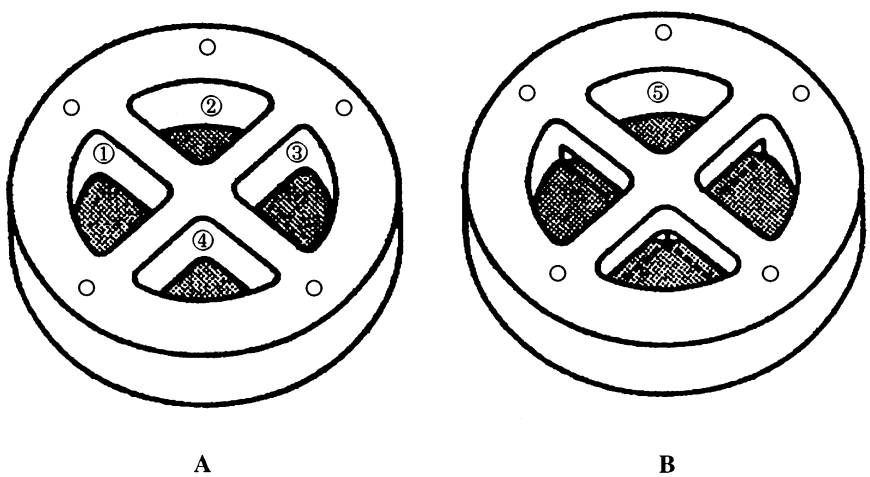

Fig. 1. Schematic representation of the Bickel et al. multi-compatment dialysis cell. (A) Juxtaposed half-cell divided in four compartments of 1.2 ml each containing the tissues solutions: (1) synovial membrane; (2) joint capsule; (3) cartilage; (4) ligament. (B) Half-cell in which the partitions are incomplete so that it is a single 5 ml compartment for the buffer (5).

2.6. Data analysis

The concentrations were expressed in micrograms of drug per gram of dried tissue (C_{dt}) as follows:

$$C_{dt} = \frac{c}{W_{dt}}$$

where W_{dt} is the weight of dried tissue in homogenized solutions of fresh tissues (mg ml^{-1}) and C is the concentration in homogenized solutions of fresh tissues (ng ml^{-1}).

2.7. Statistical analysis

The Student's paired t-test assessed the differences in (R)- and (S)-enantiomer concentrations as well as differences in their respective distributions between the articular tissues homogenates. The alpha risk was fixed at 0.05. StatPhar® version 1.9 (Faculty of Pharmaceutical Sciences, Limoges, France) was used for statistical analysis.

3. Results

The concentrations of ketoprofen enantiomers in articular tissues and buffer are presented in Table 1. There was no statistical difference between the $S(+)$ and $R(-)$-ketoprofen concentrations in any tissues ($P > 0.05$). Despite the interindividual variability in the concentrations in a given tissue, the highest concentrations of (S)- and (R)-ketoprofen were observed in the synovial membrane and the lowest concentrations in cartilage (Fig. 2). There were no significant differences in either (R)- or (S)-ketoprofen concentrations observed in the solutions of joint capsule, synovium and ligament tissues homogenates ($P > 0.05$). However, the concentrations of (R)- and (S)-ketoprofen were significantly higher in synovial membrane, ligament or joint capsule than in articular cartilage ($P < 0.05$) (Fig. 3).

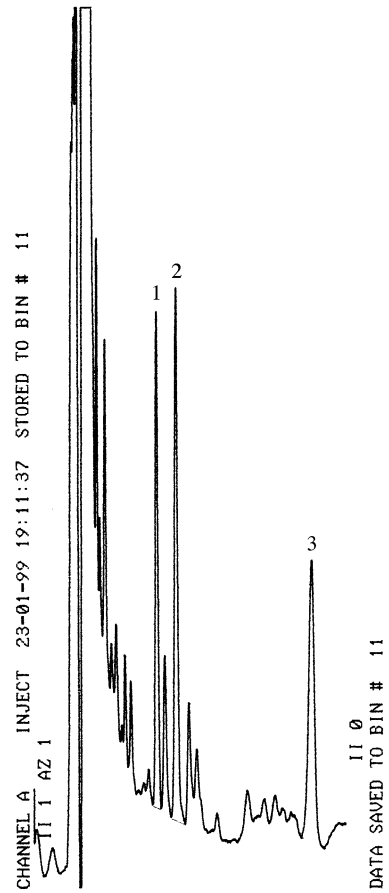

Fig. 2. Representative chromatogram: first patient's synovial membrane homogenate. Peaks 1, R-ketoprofen; peak 2, S-ketoprofen; peak 3, internal standard.

4. Discussion

The study indicates that there was a nonstereoselective affinity of ketoprofen enantiomers for articular tissues. However, there was wide interindividual variability in the concentrations of both ketoprofen enantiomers in a given tissue, especially in synovial membrane. Similar findings have already been reported in patients receiving systemic NSAID therapy [16–18]. Different factors might have contributed to the variability in the concentrations achieved in a given tissue, including variation in joint tissue pathophysiology and tissue binding of the drug [16–18]. Despite the interindividual variability in articular tissues,

Table 1
Concentrations of ketoprofen enantiomers in dried articular tissues and buffer

Patient number (joints)	Ketoprofen concentrations									
	Buffer (μg ml^{-1})		Synovial membrane (μg g^{-1})		Capsule (μg g^{-1})		Cartilage (μg g^{-1})		Ligament (μg g^{-1})	
	R(−)	*S*(+)	*R*(−)	*S*(+)	*R*(−)	*S*(+)	*R*(−)	*S*(+)	*R*(−)	*S*(+)
1 (knee)	0.57	0.56	7.91	8.11	4.09	4.16	1.34	1.22	5.65	5.89
2 (knee)	0.53	0.54	10.43	10.08	9.41	9.20	4.55	4.49	11.94	11.85
3 (hip)	0.63	0.66	4.37	4.03	9.59	10.31	4.93	5.11	5.12	5.46
4 (hip)	0.63	0.61	5.63	5.55	7.00	6.43	6.69	6.80	13.7	13.6
5 (hip)	0.49	0.44	23.59	20.42	6.43	5.48	3.96	3.80	4.71	4.16
6 (hip)	0.51	0.50	11.72	11.72	5.72	5.72	2.81	2.79	4.18	4.17
7 (hip)	0.58	0.61	5.43	5.35	2.61	2.57	2.95	2.89	3.93	3.87
8 (hip)	0.69	0.69	8.80	8.96	4.43	4.33	5.35	5.40	3.53	3.75
9 (knee)	0.59	0.54	6.31	5.62	2.34	2.11	2.31	2.11	3.38	3.09
10 (hip)	0.53	0.54	7.19	7.06	5.43	4.63	2.15	2.17	7.85	6.93
Mean (S.D.)	0.57 (0.06)	0.57 (0.08)	9.14 (5.57)	8.69 (4.76)	5.71 (2.49)	5.49 (2.62)	3.70 (1.67)	3.67 (1.75)	6.40 (3.64)	6.28 (3.61)
Range	0.49–0.69	0.41–0.69	4.37–23.59	4.03–20.42	2.34–9.59	2.11–10.31	1.34–6.69	1.22–6.80	3.38–13.7	3.09–13.6

(*R*)- and (*S*)-ketoprofen concentrated in the solutions of synovial membrane, joint capsule and ligament, while both enantiomers attained much lower concentrations in those of articular cartilage. Due to the dilution, the possible multitude of binders in a tissue and the small samples of the different tissues, the association constants of ketoprofen enantiomers (K_i) were not estimated. It therefore seems unlikely that the distribution of a drug can be predicted from tissue K_i values [8].

The articular pharmacokinetics of NSAIDs have been extensively studied in synovial fluid, i.e. the compartment close to the presumed target tissues in various rheumatic disorders [19]. Since the concentrations of NSAIDs are more sustained in synovial fluid than in plasma, the osteoarthritic articular cavity can be viewed as a physical peripheral compartment in which drug distribution results from the binding competition between articular tissues [10,19]. However, publications are too few and too disparate to distinguish NSAIDs according to their mode of distribution within the joint tissues [19]. Some molecules including ketoprofen [20], tenoxicam [16], tiaprofenic acid [17], and naproxen sodium [18,21,22] were shown to achieve higher concentrations in synovial membrane and/or entheses than in cartilage. Ketoprofen was also shown to penetrate Achille's tendon [20]. Rolf et al. [20] reported median maximal concentrations in fresh tissue of 363.9 ng g^{-1} in synovium, 83.5 ng g^{-1} in cartilage and 85.7 ng g^{-1} in meniscus in 30 patients who were given a single oral dose of 50 mg ketoprofen. Nabumetone showed a marked affinity for synovial tissue and slightly lower affinity for fibrous capsule [23]. Conversely, multiple topical applications of ketoprofen resulted in concentrations in cartilage and menisci six- to tenfold greater than those measured in synovial tissue [20]. Whether these discrepant findings may be explained by the route of administration is unknown.

NSAIDs may also interfere with bone metabolism, as reflected by their activity in certain forms of paraneoplastic hypercalcaemia and their preventive effect on heterotopic ossification [24]. The role of prostaglandin PGE_2 in osteoclastic resorption supports the hypothesis that bone also constitutes a site of action for NSAIDs [24]. Low concentrations of naproxen were also detected in bone [18]. However, the limited number of com-

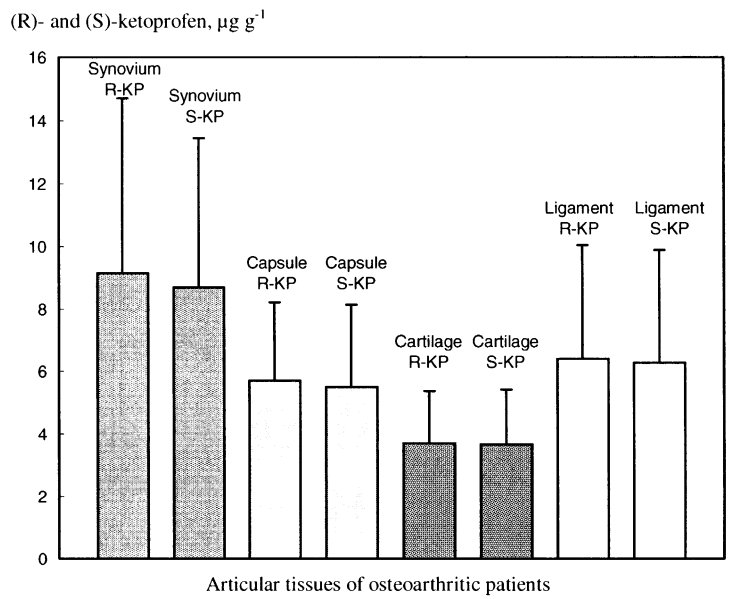

Fig. 3. Comparative distribution of *S*- and *R*-ketoprofen (KP) between the compartments of the dialysis cell expressed in microgram per gram of dried tissue (mean ± S.D.).

partments of the Bickel's model as well as the high lipid content of bones persuaded us not to perform this experiment.

Finally, in vivo data showed that systemically administered NSAIDs penetrated to a larger extent the synovium and joint capsule than joint cartilage and even cancellous bone. The heterogeneous distribution of NSAIDs in joint tissues was ascribed to differences in histopathological features of the different tissues. Cartilage is virtually devoid of blood vessels, in contrast to other joint tissues. Furthermore, inflammatory synovitis may be present in osteoarthritis and promote the diffusion of drugs into synovium.

However, the Bickel et al. in vitro model generated similar results suggesting that the modalities of distribution may be attributed to differences in ketoprofen affinity for the different constituents of joints. Furthermore, these results agree with the lack of stereoselective distribution already reported in humans.

5. Conclusion

The distribution of ketoprofen enantiomers in the Bickel et al. multi-compartment distribution model indicated that there was a non-stereoselective affinity of ketoprofen enantiomers for their potential target tissues. Both (*R*)- and (*S*)-ketoprofen concentrated in synovial membrane, joint capsule, and ligament, whereas much lower concentrations were found in articular cartilage. These data may be related to differences in ketoprofen affinity for the different tissular constituents of joints. Our results demonstrate the utility of the in vitro model of Bickel et al. since it may predict in vivo distribution of NSAIDs within joint tissues.

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